

ART 34 AMDT
13-04-2004

NL0300049

EPO - DG 1

PCT/NL03/00049

128

13.04.2004

Amended Claims

(94)

1. A eukaryotic host cell transformed with a nucleic acid construct comprising a nucleotide sequence encoding a xylose isomerase comprising an amino acid sequence
5 that has at least 70 % sequence identity with the amino acid sequence of SEQ ID NO: 1, whereby the nucleic acid construct, upon transformation of the host cell, confers to the host cell the ability to grow on xylose as carbon source.
2. A transformed host cell according to claim 1, wherein the host cell is a yeast,
10 preferably a yeast that belongs to one of the genera: *Saccharomyces*, *Kluyveromyces*, *Candida*, *Pichia*, *Schizosaccharomyces*, *Hansenula*, *Kloeckera*, *Schwannomyces*, and *Yarrowia*.
3. A transformed host cell according to claim 2, wherein the yeast belongs to one of
15 the species: *S. cerevisiae*, *S. bulderi*, *S. barnettii*, *S. exiguus*, *S. uvarum*, *S. diastaticus*, *K. lactis*, *K. marxianus*, and *K. fragilis*.
4. A transformed host cell according to claim 1, wherein the host cell is a filamentous fungus, preferably a filamentous fungus that belongs to one of the genera:
20 *Aspergillus*, *Trichoderma*, *Hemicola*, *Acremonium*, *Fusarium*, and *Penicillium*.
5. A transformed host cell according to any one of the preceding claims, whereby the nucleotide sequence encoding a xylose isomerase is operably linked to a promoter that causes sufficient expression of the xylose isomerase in the host cell, to confer to
25 the host cell the ability to isomerise xylose into xylulose.
6. A transformed host cell according to claim 6, whereby the promoter is insensitive to catabolite repression in the host cell.
- 30 7. A transformed host cell according to any one of the preceding claims, whereby the host cell comprises a genetic modification that result in a characteristic selected from the group consisting of:
(a) increase transport of xylose into the host cell;

BEST AVAILABLE COPY

AMENDED SHEET

ART 34 AMDT

13-04-2004

NE0300049

2 29

- (b) increased xylulose kinase activity;
(c) increased flux of the pentose phosphate pathway;
(d) decreased sensitivity to catabolite repression;
(e) increased tolerance to ethanol, osmolality or organic acids; and,
5 (f) reduced production of by-products.

8. A transformed host cell according to claim 7, wherein the genetic modification consist of overexpression of endogenous genes, expression of a heterologous genes, or a combination thereof, and whereby the gene is selected from the group consisting of a
10 gene encoding: a hexose or pentose transporter, an xylulose kinase; an enzyme from the pentose phosphate pathway, a glycolytic enzyme, and an ethanologenic enzymes.

9. A transformed host cell according to claim 7, wherein the genetic modification consist of the inactivation of an endogenous genes, whereby the gene is selected from
15 the group consisting of a gene encoding a hexose kinase gene, the *Saccharomyces MIG1* and *MIG2* genes and hybridising homologues thereof.

10. A transformed host cell according to any one of the preceding claims, whereby the host cell expresses one or more enzymes that confer to the host cell the ability to
20 produce lactic acid, acetic acid, succinic acid, amino acids, 1,3-propane-diol, ethylene, glycerol, β -lactam antibiotics and cephalosporins.

11. A transformed host cell according to claim 10, whereby the host cell contains a
25 genetic modification that results in decreased alcohol dehydrogenase activity.

12. A process for producing ethanol, whereby the process comprises the steps of:
(a) fermenting a medium containing a source of xylose with a transformed host cell as defined in any one of claims 1 - 9, whereby the host cell ferments xylose to ethanol,
30 and optionally,
(b) recovery of the ethanol.

BEST AVAILABLE COPY

AMENDED SHEET

ART 13-04-2004

NL0300049

330

13. A process according to claim 12, whereby the medium also contains a source of glucose.
14. A process according to claims 12 or 13, whereby the volumetric ethanol productivity is at least 0.5 g ethanol per litre per hour.
15. A process according to claims any one of claims 12 - 14, whereby the ethanol yield is at least 50 %.
- 10 16. A process for producing a fermentation product selected from the group consisting of lactic acid, acetic acid, succinic acid, amino acids, 1,3-propane-diol, ethylene, glycerol, β -lactam antibiotics and cephalosporins, whereby the process comprises the steps of:
- 15 (a) fermenting a medium containing a source of xylose with a transformed host cell as defined in claims 10 or 11, whereby the host cell ferments xylose to the fermentation product, and optionally,
- (b) recovery of the fermentation product.
- 20 17. A process according to claim 16, whereby the medium also contains a source of glucose.

BEST AVAILABLE COPY

AMENDED SHEET